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Strong Linkage Disequilibrium and Haplotype Analysis in Japanese Pedigrees With Machado-Joseph Disease

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To identify the markers tightly linked to Machado-Joseph disease (MJD) and to investigate whether a limited number of ancestral chromosomes are shared by Japanese MJD pedigrees, a detailed linkage analysis employing D14S55, D14S48, D14S67, D14S291, D14S280, AFM343vf1, D14S81, D14S265, D14S62, and D14S65 was performed. The results of multipoint linkage analysis as well as detection of critical recombination events indicate that the gene for MJD is localized in a 4-cM region between D14S280–D14S81. We found strong linkage disequilibria at AFM343vf1 and D14S81, and association of a few common haplotypes with MJD. These results indicate that there is an obvious founder effect in Japanese MJD and suggest the possibility of the existence of predisposing haplotypes which are prone to expansions of CAG repeats. © 1996 Wiley-Liss, Inc.

KEY WORDS: multipoint linkage analysis, AFM343vf1, CAG repeat, founder effect, allelic association

INTRODUCTION

Machado-Joseph disease is an autosomal-dominant neurodegenerative disorder characterized by cerebellar ataxia accompanied by various combinations of pyramidal signs, nystagmus, progressive ophthalmoplegia, dystonia, facial fasciculation, bulging eyes, and peripheral amyotrophy with onset usually in the fourth and fifth decades [Nakano et al., 1972; Woods and Schaumburg, 1972; Rosenberg et al., 1976; Coutinho and Andrade, 1978; Lima and Coutinho, 1980]. The prevalence rate of MJD is as high as 1 in 4,000 in individuals of Azorean descent [Sequeiros and Coutinho, 1993]. From a nationwide survey performed in 1990 in Japan, the prevalence rate of MJD in Japanese has been reported to be 0.2 in 100,000 [Hirayama et al., 1994].

Using a systematic genome search we mapped the MJD gene to 14q24.3–32.1 by linkage analysis in five Japanese MJD families [Takiyama et al., 1993]. This MJD gene location has subsequently been confirmed by other groups in Azorean as well as Japanese MJD pedigrees [Sequeiros et al., 1994; St. George-Hyslop et al., 1994; Sasaki et al., 1995a]. Recently, Kawaguchi et al. [1994] discovered that MJD is caused by unstable expansion of CAG repeats of the *MJD1* gene at 14q32.1.

Although initially reported cases of MJD were found in families of Portuguese-Azorean ancestry [Nakano et al., 1972; Woods and Schaumburg, 1972; Rosenburg et al., 1976; Coutinho and Andrade, 1978], MJD has also been described in other ethnic populations, including Japanese [Sakai et al., 1983; Yuasa et al., 1986; Kitamura et al., 1989; Takiyama et al., 1989], Chinese

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[Wang et al., 1990], Indian [Bharucha et al., 1986; Jain and Maheshwari, 1990], Black [Healton et al., 1980], Italian [Livingstone and Sequeiros, 1984; Suite et al., 1986], Spanish [Pou-Serradell et al., 1987], non-Azorean Portuguese [Lima and Coutinho, 1980] and Brazilian [Fowler, 1984]. Since some of these ethnic populations had some connections to the Portuguese in the past, one can hypothesize that the MJD mutation originated in a Portuguese population and was spread to other ethnic populations by Portuguese emigration or by Portuguese navigators. If this is the case, one can hypothesize that a limited number of haplotypes are shared by MJD chromosomes in various ethnic populations.

Recent investigations have revealed the instability and expansion of trinucleotide repeats in Huntington's disease (HD) [Hungtington's Disease Collaborative Research Group, 1993], spinocerebellar ataxia type 1 (SCA1) [Orr et al., 1993], and fragile X syndrome [Knight et al., 1993, 1994; Kremer et al., 1991; Oberle et al., 1991; Verkerk et al., 1991; Yu et al., 1991]. These results also raise the intriguing possibility that there are predisposing chromosomes which are prone to repeat expansion. Therefore, it is crucial to determine whether a limited number of founder MJD chromosomes exist not only for the investigation of the origin of MJD mutations but also for the investigation of the possibility of the existence of *cis*-acting elements causing size instability of the CAG repeats.

Results of previous analyses of Japanese MJD pedigrees suggest the possibility of the presence of a founder in these pedigrees [Sasaki et al., 1995a; Takiyama et al., 1995]. In the present study, we performed further linkage analysis to identify markers tightly linked to MJD, and initiated a collaborative research effort to investigate haplotypes in a large number of Japanese MJD pedigrees using these newly identified markers tightly linked to MJD.

MATERIALS AND METHODS

Pedigrees

In the present study, we analyzed 30 pedigrees, eight collected at Niigata University and Jichi Medical School for further linkage analysis to identify markers tightly linked to MJD, and 22 collected at Hokkaido University for detailed haplotype analysis using the tightly linked markers.

After the *MJD1* gene was discovered [Kawaguchi et al., 1994], the CAG repeats of the *MJD1* gene were analyzed in 32 Japanese pedigrees, each of which included at least one individual with clinically diagnosed MJD. Expanded CAG repeats of the *MJD1* gene were identified in eight of eight [Takiyama et al., 1993, 1995] and 22 of 24 [Sasaki et al., 1995b] of these pedigrees. The two pedigrees (P55 and P57 [Sasaki et al., 1995b]) in which no *MJD1* gene CAG repeat expansion was detected were not included in the present study.

Microsatellite Markers Used in the Present Study

We used D14S55, D14S48, D14S67, D14S291, D14S280, AFM343vf1, D14S81, D14S265, D14S62, and D14S65 in the present study. Blood samples were obtained from patients and unaffected family members, and from 126 unrelated normal controls living in the Niigata, Tochigi, or Hokkaido areas of Japan, with informed consent. High molecular weight genomic DNA was extracted from either peripheral blood leukocytes or lymphoblastoid cell lines [Maniatis et al., 1989]. Analyses of polymorphisms were performed by polymerase chain reaction (PCR) as described elsewhere [Weber and May, 1989]. Allele sizes were determined using M13 sequence ladders as described elsewhere [Takiyama et al., 1995]. The codes of alleles for D14S291, D14S280, AFM343vf1, and D14S81 are as follows: D14S291, 1, 219 bp; and 2, 217 bp; D14S280, 1, 247 bp; 2, 245 bp; 3, 243 bp; 4, 241 bp; 5, 239 bp; 6, 237 bp; 7, 235 bp; 8, 233 bp; 9, 231 bp; and 10, 229 bp; AFM343vf1, 1, 140 bp; 2, 138 bp; 3, 136 bp; 4, 134 bp; 5, 132 bp; 6, 130 bp; and 7, 128 bp; and D14S81, 1, 192 bp; 2, 190 bp; 3, 188 bp; 4, 186 bp; 5, 184 bp; 6, 182 bp; 7, 180 bp; 8, 178 bp; 9, 176 bp; 10, 174 bp; 11, 172 bp; 12, 170 bp; 13, 168 bp; and 14, 166 bp. To allow use of the same codes for the comparison of alleles and haplotypes, the coding system in the present study was different from that described in a previous report [Sasaki et al., 1995a].

Linkage Analysis

Detailed linkage analysis was performed on the eight pedigrees collected at Niigata University and Jichi Medical School. Results of linkage analyses of the 22 pedigrees collected at Hokkaido University were recently published elsewhere [Sasaki et al., 1995a]. Two-point lod scores and multipoint lod scores were calculated using the MLINK program of the LINKAGE package, version 5.03 [Lathrop and Lalouel, 1984], as described previously [Takiyama et al., 1993]. Multipoint linkage analysis was performed using the LINKMAP program of the LINKAGE package. Ten liability classes were used for the calculation of pairwise lod scores, based on the cumulative risk curve calculated from age-of-onset (in years) in the families described here (class 1: ages 0–15, 0.04; class 2: ages 16–20, 0.18; class 3: ages 21–25, 0.29; class 4: ages 26–30, 0.40; class 5: ages 31–35, 0.51; class 6: ages 36–40, 0.62; class 7: ages 41–45, 0.73; class 8: ages 46–50, 0.85; class 9: ages 51–55, 0.96; class 10: ages >56, 1.00). For the calculation of multipoint lod scores, we used six liability classes (in years) to reduce the computation time (class 1: ages 0–15, 0.04; class 2: ages 16–25, 0.23; class 3: ages 26–35, 0.46; class 4: ages 36–45, 0.68; class 5: ages 46–55, 0.90; class 6: ages >56, 1.00).

Linkage Disequilibrium and Extended Haplotype Analysis

Taking account of the results of the preliminary study of eight pedigrees using the 10 microsatellite markers listed above, we selected D14S291, D14S280, AFM343vf1, and D14S81 to compare the affected haplotypes among the families. For comparison of haplotypes, the 22 MJD pedigrees collected at Hokkaido University were included in addition to the eight MJD

pedigrees collected at Niigata University and Jichi Medical School. Partial haplotypes of the 22 pedigrees, except for AFM343vf1, were described elsewhere [Sasaki et al., 1995a]. Thirty-one haplotypes were determined from analysis of the 30 pedigrees, since the pedigree Yz included 2 affected siblings carrying different haplotypes (Yz[1] and Yz[2]) due to an obligate recombination event.

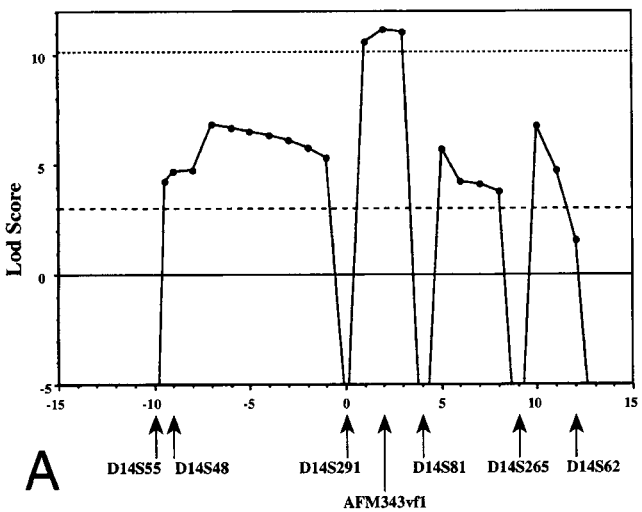
To determine whether a single allele was present significantly more frequently on the disease chromosomes, the allele frequencies of the MJD chromosomes and those of normal controls were compared using the χ^2 test. For comparison of frequencies of haplotypes, we determined 34 extended haplotypes of unaffected married-in individuals in the MJD pedigrees. To assess degree of linkage disequilibrium, the parameter δ, which was originally defined by Bengtsson and Thomson [1981] and has been demonstrated to be a superior measure for determining the likely location of a disease locus [Devlin and Risch, 1995], was calculated.

RESULTS

Linkage Analysis

In the linkage analysis of the eight MJD pedigrees, the highest pairwise maximal lod scores of 10.0 and 9.2 were obtained at a recombination fraction of 0 at D14S67 and D14S48, respectively. Similarly high lod scores of 9.2 and 9.0 were obtained at AFM343vf1 (with a recombination fraction of 0) and D14S280 (with a recombination fraction of 0.01), respectively. The results of multipoint linkage analyses are shown in Figure 1A. The highest lod score of 11.2 was obtained at AFM343vf1. In the interval between D14S55–D14S291, the highest lod score of 7.0 was obtained 7 cM centromeric to D14S291. The 95% confidence interval was obtained at an approximately 2-cM interval near AFM343vf1 (Fig. 1A).

As shown in Figures 1B and 2, two obligate recombination events were detected between D14S291–AFM343vf1, and another obligate recombination event was detected between AFM343vf1–D14S81 (we also identified one recombination event at D14S291), indicating that the MJD gene is likely to be located in the 4-cM interval between D14S291–D14S81.



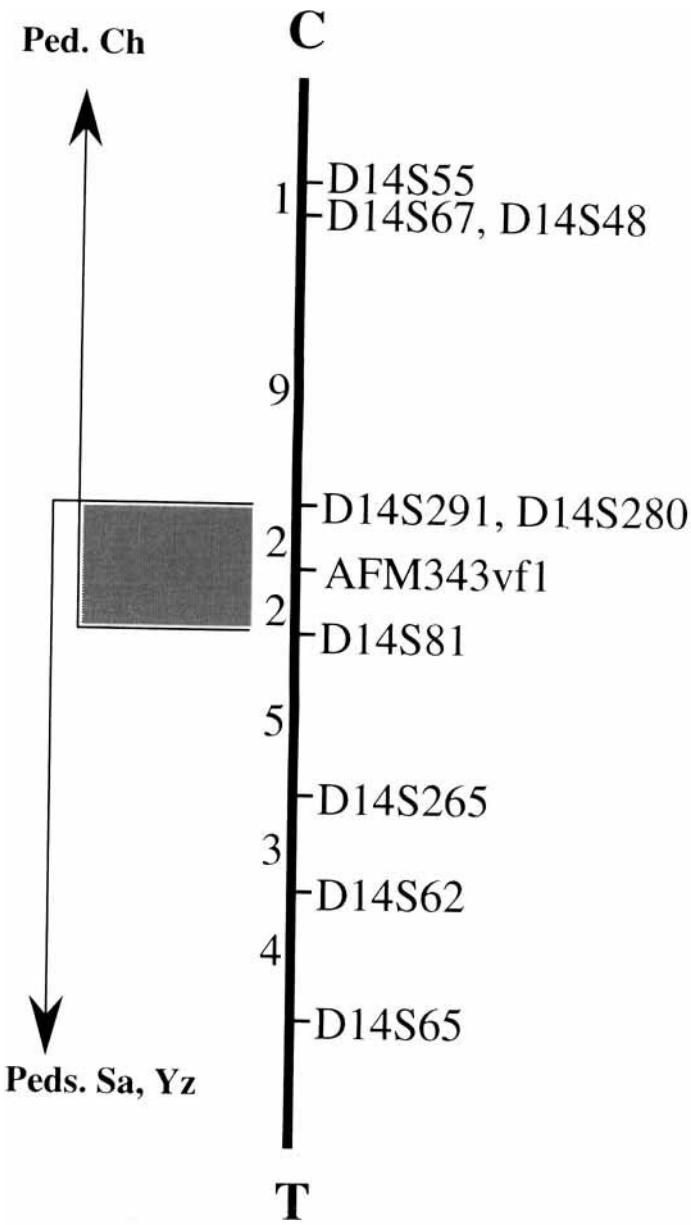


Fig. 1. **A:** Multipoint linkage analysis was performed using D14S55, D14S48, D14S291, AFM343vf1, D14S81, D14S265, and D14S62. Highest lod score of 11.2 was obtained at AFM343vf1. The 95% confidence interval was obtained at an approximately 2-cM interval between D14S291–D14S81. **B:** Genetic regional maps of chromosome 14q. Two obligate recombination events at D14S291 and one obligate recombination at D14S81 indicate that the *MJD1* gene is likely to be located in the interval between D14S291–D14S81.

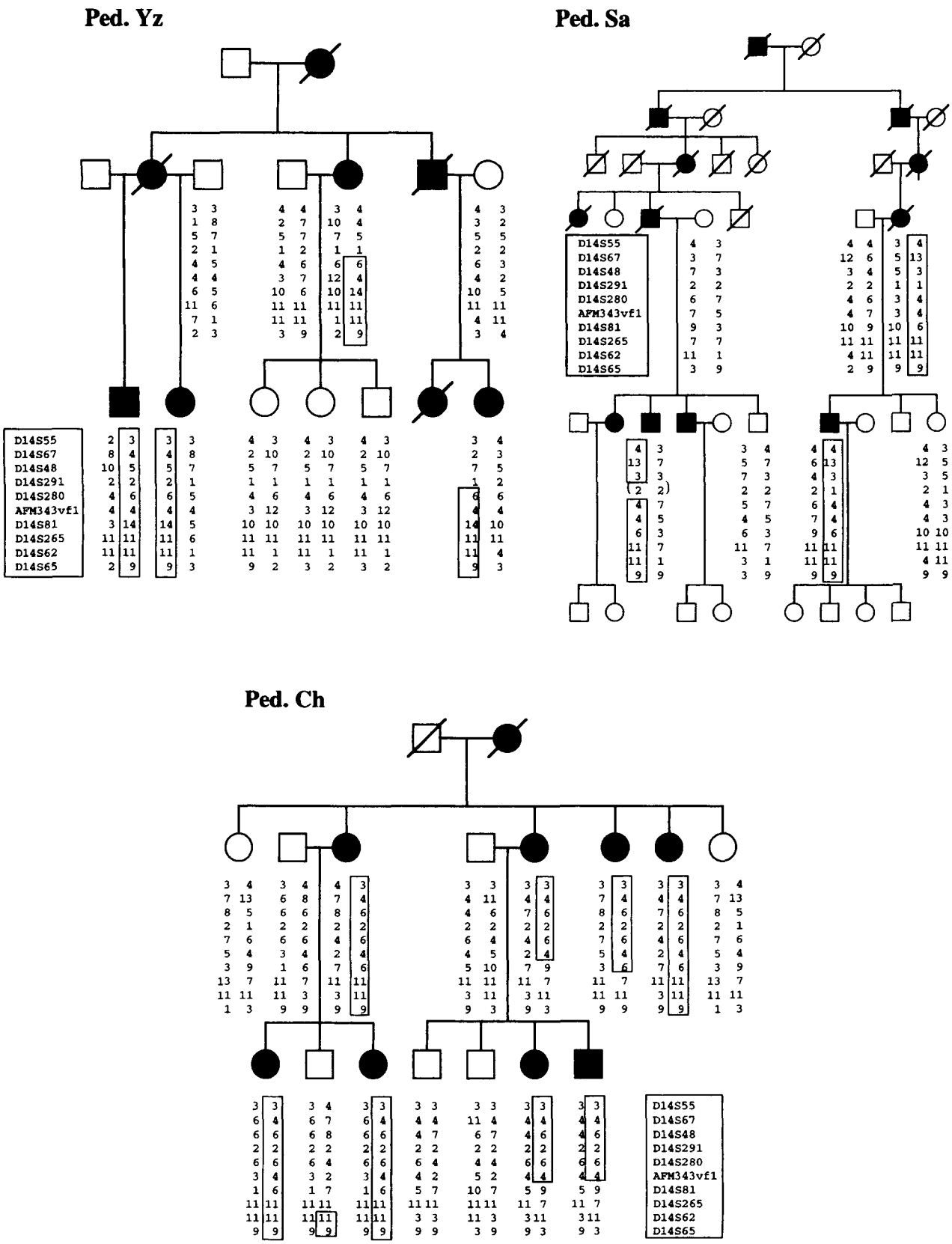


Fig. 2. We detected a total of three obligate recombination events in affected individuals, two between D14S291–AFM343vf1 in Ped. Yz and Ped. Sa, and the other between D14S291–D14S81 in Ped. Ch.

Haplotype Analysis and Linkage Disequilibrium

The results of the preliminary study of eight pedigrees using the 10 microsatellite markers suggested a linkage disequilibrium at AFM343vf1. To investigate the possibility of linkage disequilibrium in Japanese MJD pedigrees, we examined the haplotypes using a series of markers (D14S291, D14S280, AFM343vf1, and D14S81) spanning a genetic distance of 4 cM. In this analysis, we included the 22 Japanese pedigrees which were collected and analyzed at Hokkaido University, in addition to the eight Japanese MJD pedigrees collected at Niigata University and Jichi Medical School. As summarized in Table I, we determined unambiguously 31 MJD haplotypes at the D14S291, D14S280, AFM343vf1, and D14S81 loci. We identified two closely related haplotypes in branches of Ped. Yz. We also determined 34 haplotypes of unaffected married-in individuals in these pedigrees.

Allele frequencies determined for each locus based on the haplotypes linked to MJD were compared with those in normal individuals, and we found significant linkage disequilibria at AFM343vf1 ($\delta = 0.708$, $\chi^2 = 16.8$, $P < 0.0001$) and D14S81 ($\delta = 0.505$, $\chi^2 = 9.7$, $P = 0.018$), as shown in Table II. Of particular interest is AFM343vf1, where 83.9% of the MJD haplotypes displayed allele 4, while allele 4 was found at this locus in only 33.3% of 252 normal controls. A similar phenomenon was identified at D14S81, where alleles 3 and 6 were found more commonly (71%) in MJD haplotypes than in those of normal controls (41.4%).

We also found significant differences in the frequencies of Japanese MJD haplotypes compared to those determined for 34 married-in unaffected individuals. The frequencies of the 6-4-3 and 4-4-6 haplotypes at D14S280, AFM343vf1, and D14S81 were 22.6% and 16.1%, respectively, which is in striking contrast to the 0% and 5.9% frequencies of these haplotypes in the 34 married-in unaffected individuals ($\chi^2 = 11.5$, $P < 0.01$), respectively. Interestingly, most of the MJD pedigrees showing one of these 6-4-3 and 4-4-6 haplotypes at D14S280, AFM343vf1, and D14S81 originated in Northern Japan, including Toyama and Niigata prefectures which are located adjacent to each other (Fig. 3). These data strongly support the hypothesis of the presence of a founder effect in Japanese MJD pedigrees, especially for those originating in Toyama and Niigata prefectures.

DISCUSSION

Results of the multipoint linkage analysis as well as detection of critical recombination events between D14S291–D14S81 in MJD patients strongly indicate that the *MJD1* gene is located in the 4-cM interval between D14S291–D14S81, which is consistent with recently published findings obtained in studies on Caucasian [Sequeiros et al., 1994; St. George-Hyslop

TABLE I. D14S291-D14S280-AFM343vf1-D14S81 Haplotypes Carrying the MJD Gene*

Pedigree	No. of patients	Ancestors from	Haplotype						
			D14S291	-	D14S280	-	AFM343vf1	-	D14S81
P62	3	Fukuoka	1	-	6	-	3	-	6
P53	6	Tochigi	2	-	6	-	3	-	6
P56	2	Nagasaki	2	-	4	-	4	-	3
P3	3	Unknown	1	-	6	-	4	-	3
P34	4	Niigata	1	-	6	-	4	-	3
P1	12	Toyama	2	-	6	-	4	-	3
P17	5	Toyama	2	-	6	-	4	-	3
P30	4	Toyama	2	-	6	-	4	-	3
P37	5	Niigata	2	-	6	-	4	-	3
P54	3	Unknown	2	-	6	-	4	-	3
Ped. Na	2	Niigata	1	-	4	-	4	-	6
Ped. Sa	2	Niigata	1	-	4	-	4	-	6
P19	2	Niigata	1	-	4	-	4	-	6
Ped. On	3	Niigata	2	-	4	-	4	-	6
Ped. Yo	2	Niigata	2	-	4	-	4	-	6
P31	2	Tokyo	1	-	6	-	4	-	6
Ped. Ch	5	Tochigi	2	-	6	-	4	-	6
P8	4	Toyama	2	-	6	-	4	-	6
P58	4	Yamagata	2	-	6	-	4	-	6
Ped. Yg	3	Niigata	2	-	7	-	4	-	6
P45	2	Yamagata	2	-	6	-	4	-	9
P5	2	Osaka	2	-	6	-	4	-	10
P40	1	Miyagi	2	-	6	-	4	-	10
P15	3	Fukushima	2	-	7	-	4	-	10
Ped. Hi	21	Tochigi	1	-	10	-	4	-	10
P44	1	Aomori	1	-	5	-	4	-	11
Ped. Yz [1]	2	Tokyo	1	-	6	-	4	-	14
Ped. Yz [2]	2	Tokyo	2	-	6	-	4	-	14
P18	6	Fukushima	1	-	4	-	5	-	10
P43	3	Akita	2	-	6	-	7	-	10
P7	3	Unknown	1	-	6	-	7	-	10

*Common haplotypes of 6-4-3 and 4-4-6 are boxed.

TABLE II. Linkage Disequilibrium Between MJD and 14q Markers

Locus	Allele	χ^2	p	δ
D14S291	1	0.845	0.358	−0.250
	2	0.751	0.386	0.118
D14S280	6	0.401	0.527	0.146
AFM343vf1	4	16.825	<0.0001	0.708
D14S81	3 or 6	9.731	0.018	0.505

et al., 1994] and Japanese [Sasaki et al., 1995a] MJD patients. Recently, Twist et al. [1995] reported that the *MJD1* gene was located in an 11-cM interval between D14S68–AFM343vf1, based on recombination events involving these loci. Taken together, these results suggest that the *MJD1* gene is most likely located between D14S291–AFM343vf1, probably close to AFM343vf1. These results indicate that haplotype analysis employing AFM343vf1 and flanking markers including D14S291/D14S280 and D14S81 is ideal for linkage disequilibrium studies.

Interestingly, we found that 26 of 31 MJD-linked haplotypes (83.9%) displayed the 4 allele at AFM343vf1 ($\chi^2 = 16.8$, $P < 0.001$). Furthermore, alleles 3 and 5, which differ in size by only one repeat unit from the 4 allele, were also detected at AFM343vf1 in three haplotypes. As the gain or loss of one repeat unit has been shown to be a relatively common phenomenon, it is possible that these alleles (3 and 5) are derived from the 4 allele by either gain or loss of one repeat unit. A similarly high frequency of the 6 or 3 allele at D14S81 ($\chi^2 = 9.7$, $P = 0.018$), although lower than the 3, 4, and 5 allele frequencies at AFM343vf1, was demonstrated. The strong allelic association at AFM343vf1 and D14S81 indicates a linkage disequilibrium. The δ value for the AFM343vf1 locus was also exceedingly high (0.708), which suggests that of the markers used in the present study, AFM343vf1 is the closest to the disease

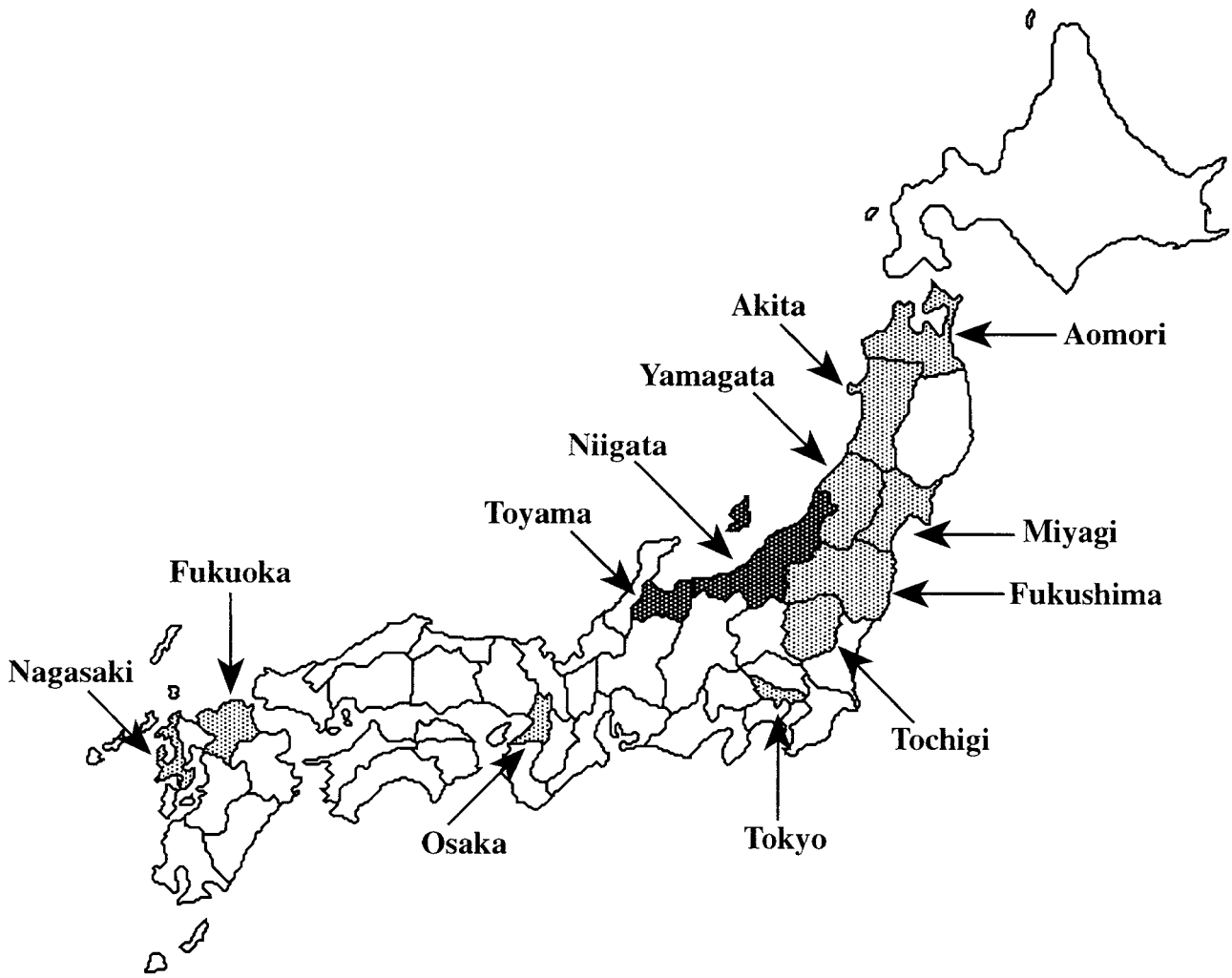


Fig. 3. Distribution of the 30 Japanese MJD pedigrees in Japan. Prefectures where MJD pedigrees originated are shown by ▦ and ▩. Prefectures where MJD pedigrees including individuals carrying either of the common 6-4-3 and 4-4-6 haplotypes at D14S291, AFM343vf1, and D14S81 originated are shown by ▩.

gene. Through extended haplotype analysis, we were able to determine 31 haplotypes linked to MJD. Comparison of these haplotypes revealed that the two common haplotypes (6-4-3 and 4-4-6) at D14S280, AFM343vf1, and D14S81 were shared among MJD patients.

Taken together, the results strongly suggest the presence of a founder effect in Japanese MJD. It is well-known that MJD is common in Toyama and Niigata prefectures, and that most, if not all, MJD pedigrees originating in these prefectures display common haplotypes (Table II). These data further indicate that there is a founder effect in Japanese MJD.

A number of recent investigations of Huntington's disease [MacDonald et al., 1992; Squitieri et al., 1994], myotonic dystrophy [Imbert et al., 1993; Rubinsztein et al., 1994], and fragile X syndrome [Richards et al., 1992; Oudet et al., 1993; Kunst and Warren, 1994] have revealed nonrandom association between the expanded alleles and genotypes for the most tightly-linked flanking markers, suggesting the presence of either a limited number of "founder chromosomes," or alternatively, predisposing haplotypes which are prone to expansion of trinucleotide repeats. Although results of the present study suggest the presence of a founder effect, there remains the possibility of the presence of predisposing haplotypes prone to expansion of CAG repeats. To investigate the fundamental issues of size instability of the CAG repeats of the *MJD1* gene, extended haplotype analysis of a large number of MJD patients of various ethnic backgrounds, using flanking markers and preferably intragenic polymorphic loci, will be required.

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